Fifth Quarterly Report

Date of Report: *September 14, 2009*

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Prepared for: DOT-PHMSA

Project Title: Effect of Concentration and Temperature of Ethanol in Fuel Blends on Microbial and Stress Corrosion Cracking of High-Strength Steels

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For quarterly period ending: *September 14, 2009*

Technical Status Section:

Technical efforts for this quarter have included establishment of industrial contacts, field visits and sampling from ethanol infrastructure, microbiological analysis of field samples, electrochemical testing, preparation of cyclic loading equipment, sectioning and preparation of tensile and compact tension specimens, baseline tensile testing of steel material, and completion of the first annual review meeting.

Field Assessment and Microbiological Characterization

Field Assessment: E10 Aboveground Storage Tanks at Fueling Stations

Black films have been observed on the external surfaces of E10 aboveground storage tanks as shown in [Figure 1]. The films were found on a variety of surfaces (painted and bare metal) near vents or joints on the AST systems. Adjacent AST's containing fuels without ethanol were free of the films. Sampling was conducted of these biofilms from AST's at several different fueling locations. The samples were collected for microbiological analysis to provide insight into the types of microbes that may affect MIC of steel in ethanol fuel blend service.

Biological analyses were conducted to determine the microbial species present and to investigate how this microbial diversity may affect corrosion behavior of the steel. 16S rRNA gene sequencing indicated a microbial community of relatively low diversity. Continued analysis of 16S rRNA gene data as well as cultivation experiments are currently underway.

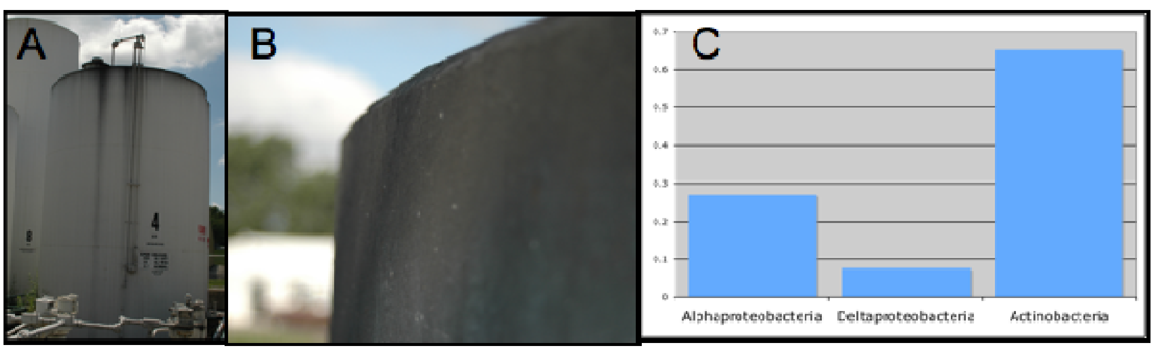

Figure 1: A and B – images of black biofilms on the external surface of storage tanks containing ethanol fuel blends. C – Microbial community of black biofilms as determined by 16S rRNA gene sequencing.

Field Assessment: Fuel Grade Ethanol Production Filters

Filters from an ethanol fuel production facility have been collected for microbiological analyses. The filters were used to remove debris from the final fuel-grade ethanol product before shipment via truck.

Samples taken from the filters have been analyzed via 16S rRNA gene sequencing [Figure 2]. 16S rRNA gene sequencing has identified spore-forming bacteria. 16S rRNA gene sequence analysis will continue to characterize the microbial community in the ethanol fuel filter. Cultivation experiments are underway using media recovered from the filters. Viable organisms have been cultivated from the ethanol fuel filters.

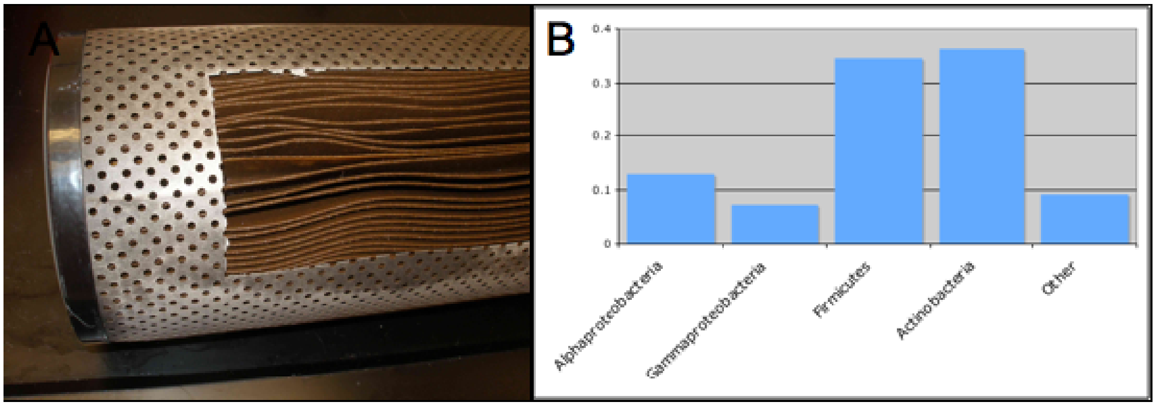

Figure 2: A – image of fuel grade ethanol filter unit (the external cover has been removed to reveal the filter). B – microbial community from a fuel grade ethanol filter as determined by 16S rRNA gene sequencing.

The identification of viable microbes isolated from ethanol fuel blend infrastructure indicates that corrosion-causing microbes may survive harsh conditions and thrive when the environment changes resulting in enhanced corrosion, which may occur during batch flow of various fuels.

Field Assessment: MIC/eSCC AST Failure

Samples from a steel tank failure experiencing a combination of microbiologically induced corrosion (MIC) and ethanol stress corrosion cracking (eSCC) have been received. MIC is believed to have originated during previous service conditions resulting in the pit formation. Subsequent containment of E10 fuel may have resulted in the initiation of eSCC at stress concentrations associated with the microbiological pitting. A more complete analysis and report of this incident will be included in the next report.

Bacillus species spores and manganese oxidation

Spores are a highly protective mode of life that some species of Bacteria employ to survive harsh conditions. The spores of some Bacillus sp. have the ability to promote the oxidation of manganese [Figure 3]. These spores may promote MIC, and investigations to determine if spores can oxidize manganese and promote corrosion in ethanol fuel blend environments has begun.

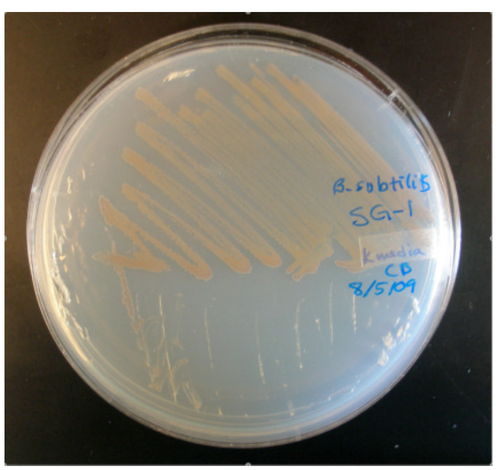

Figure 3: Manganese oxides (brown streaks) that have resulted from the spore-mediated oxidation of manganese. The ability for spores to oxidize manganese in ethanol fuel blend environments and the potential affect of this oxidation on corrosion are being investigated.

Electrochemical Evaluation of Steel U-bend Specimen

Electrochemical testing of U-bend specimens in ethanol solution containing varying amounts of water, each with 19.4 mg L^{-1} Cl^- (no Cl^- in pure ethanol), as reported previously in static air, was extended to nitrogen purged and oxygen purged conditions at ambient temperature. The open circuit potentials are the most noble for pure ethanol, followed by ethanol containing 1, 3, 5, 7 and 10 pct. water in all the three cases as shown in [Figures 1, 2 and 3]. The order of the polarization resistance (R_p) for these solutions also shows a similar trend, with pure ethanol showing the highest resistance (most noble OCP) and the solution containing 10 pct. water showing the least (most active OCP). The double layer capacitance values show an exactly reverse trend. [Figures 4, 5] show the variation of polarization resistance and double layer capacitance as a function of time in static air. Under deaerated and O_2 purged conditions, the trend of these interfacial parameters was similar.

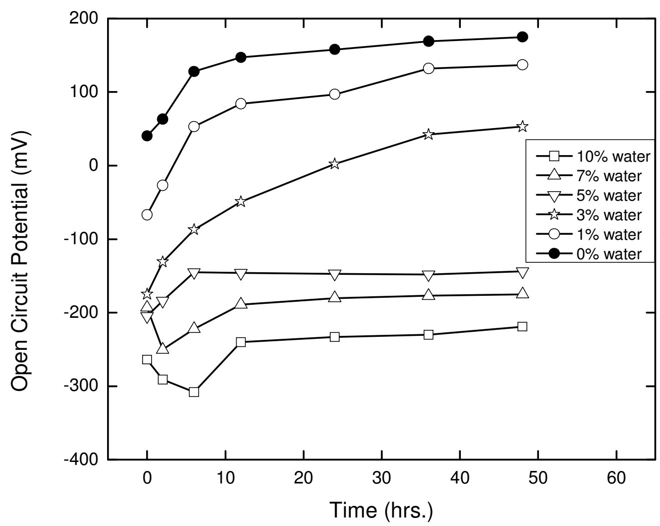

Figure 1: OCP for deaerated solution

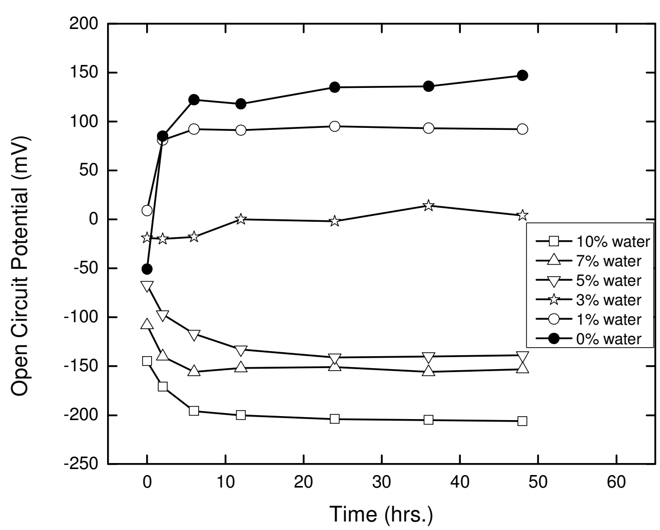

Figure 2: OCP for solution with static air

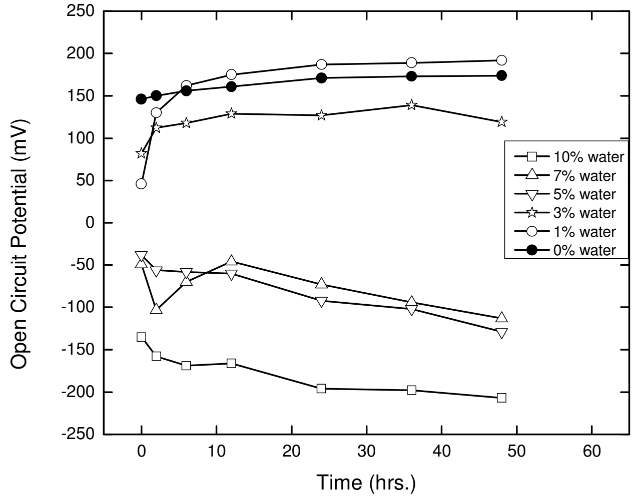

Figure 3: OCP for oxygen purged solution

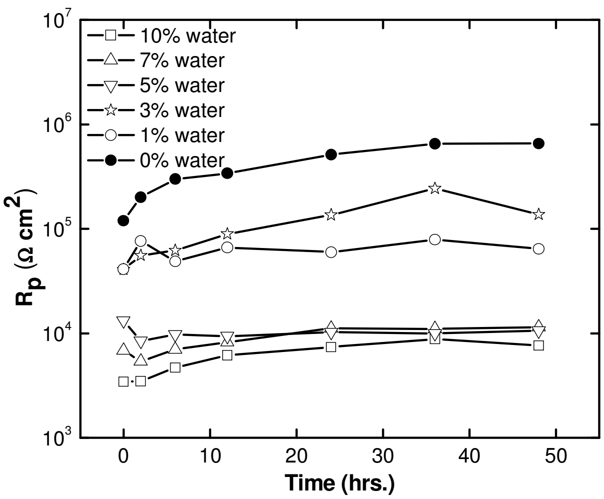

Figure 4: Polarization resistance for solution with static air

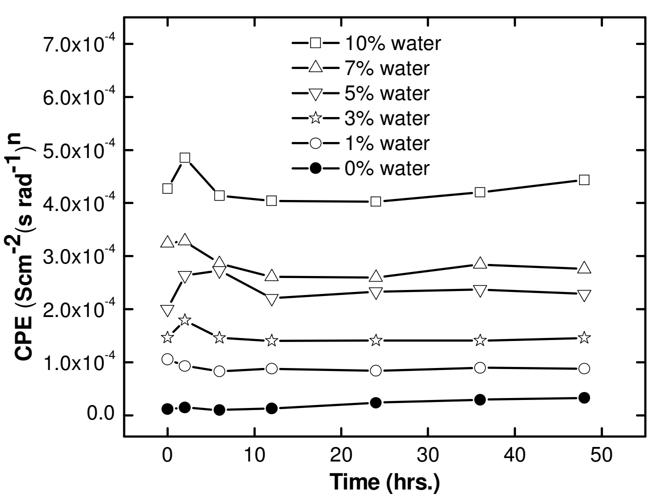

Figure 5: Double layer capacitance for solution with static air

[Figure 6] shows the variation of R_p as a function of water content in ethanol under different aeration conditions. The order of R_p is deaerated>static air>O_2 purged.

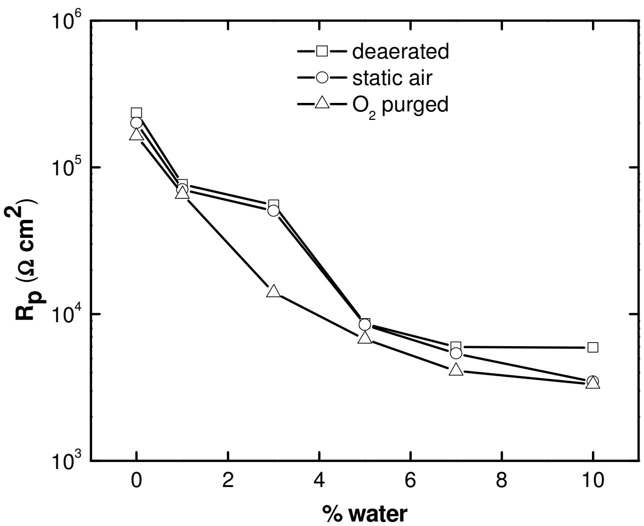

Figure 6: Polarization resistance at 2 hrs. after immersion

[Figure 7] shows the Tafel curves for ethanol containing 10 pct. water and 19.4 mg L^{-1} Cl^- in deaerated, under static air and O_2 purged solutions. It can be clearly seen from the figure that both cathodic and anodic branches of the curves seem to be affected by aeration with the corrosion rate following the order, O_2 purged>static air>deaerated.

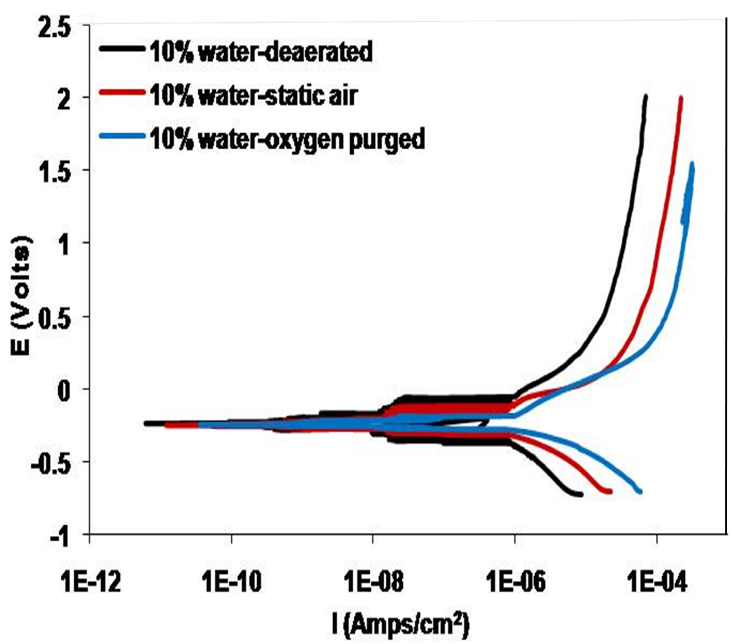

Figure 7: Tafel curve at 48 hrs. after immersion

[Figure 8] shows the Tafel curves for pure ethanol and pure ethanol containing 19.4 mg L^{-1} Cl^{-1} and 3 pct. and 10 pct. water in static air. The cathodic branches of the curves do not seem to be affected much by the varying water content, whereas the anodic branches of the curves do appear to be affected with the anodic branches shifting to the higher current side as the water content is increased. The passivation current density follows the order, 10 pct. >3 pct. > 0 pct.

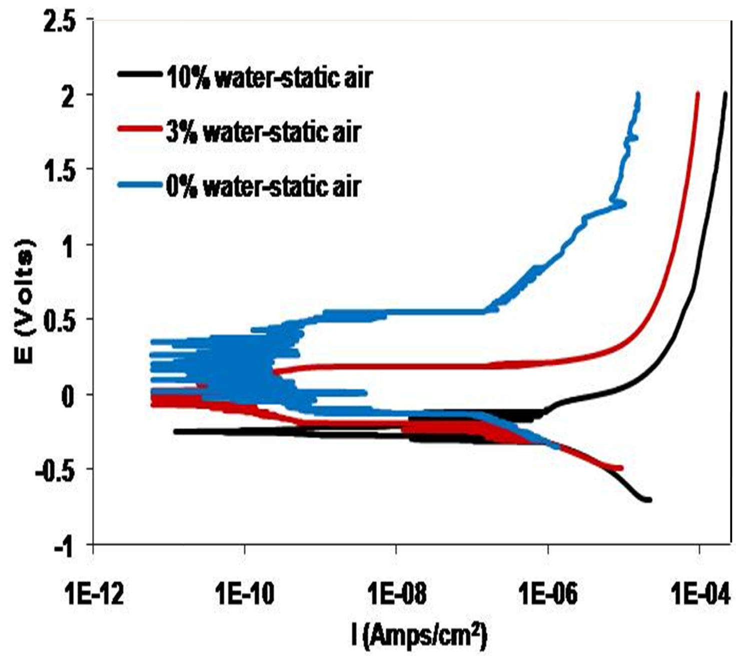

Figure 8: Tafel curve at 48 hrs. after immersion

Low Cycle Bend Testing for MIC/SCC Evaluation

Objectives for test have been determined to be:

- Screen electrochemical and microbiological environmental variables for fatigue crack growth rate (FCGR) testing
- Evaluate localized corrosive attack and fatigue crack initiation and growth due to MIC in ethanol and ethanol fuel environments
  - Characterize pitting and cracking behavior in different environments
  - Investigate environmental pitting and cracking mechanism(s)

The test procedure will operate in three phase:

Incubation: The test specimen will be subjected to a corrosive environment and inoculated with microbiological contaminates (approximately two weeks)

Loading: The test specimen with attached microbiological growth will be transferred to a loading chamber containing an identical environment and subjected to low cycle fatigue loading (two weeks)

Evaluation: The test specimen will be removed from the chamber. Biological activity and any incurred surface damage on the specimen will be evaluated
A specimen geometry has been selected as shown in [Figure 9].

The test specimens will be machined according the drawings shown in [Figure 9]:

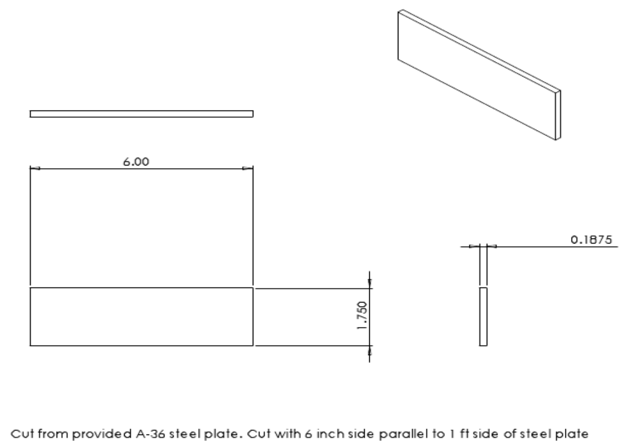

Figure 9: Bend Specimen Geometry, units in inches.

The initial testing matrix of 10 different electrochemical and microbiological environments is found in [Table 1]. Preliminary microbiological test have evaluated the highlighted environments.

Table 1: Initial testing matrix for low cycle bend testing

	Environment	**Aeration**	**Microbial Presence**
1	E10 and Water (2-phase)	Static	Sterile
2	E10 and Water (2-phase)	Static	Inoculated
3	E10 and Water (2-phase)	Nitrogen purged	Sterile
4	E10 and Water (2-phase)	Nitrogen purged	Inoculated
5	E85 and Water (2-phase)	Static	Sterile
6	E85 and Water (2-phase)	Static	Inoculated
7	E85 and Water (2-phase)	Nitrogen purged	Sterile
8	E85 and Water (2-phase)	Nitrogen purged	Inoculated
9	Ethanol w/ 10% water	Static	Sterile
10	Ethanol w/ 10% water	Static	Inoculated w/ spores

Table 2: Optional testing matrix for evaluation of spore forming microbes

	Environment	**Aeration**	**Microbial Presence**
1	Pure Ethanol	Static	Sterile
2	Pure Ethanol	Static	Inoculated w/ spores
3	Ethanol w/ 1% water	Static	Sterile
4	Ethanol w/ 1% water	Static	Inoculated w/ spores
5	Ethanol w/ 5% water	Static	Sterile
6	Ethanol w/ 5% water	Static	Inoculated w/ spores
7	SFGE	Static	Sterile
8	SFGE	Static	Inoculated w/ spores
9	Gasoline	Static	Sterile
10	Gasoline	Static	Inoculated w/ spores

Loading parameters have been refined. Loading will operate using:

- Ideal Wave form: Sinusoidal
- Repeated Stress Cycle:
  $\sigma_{max} \neq \sigma_{min}$
- σ_{max} and σ_{min} are both tension
- Stress Ratio (R): 0.5
- Frequency: 0.1 Hz
- Low cycle fatigue region: $N \approx 10^5$

Stress on the upper most fiber of the specimen between the two specimen supports will cycle according to [Figure 10].

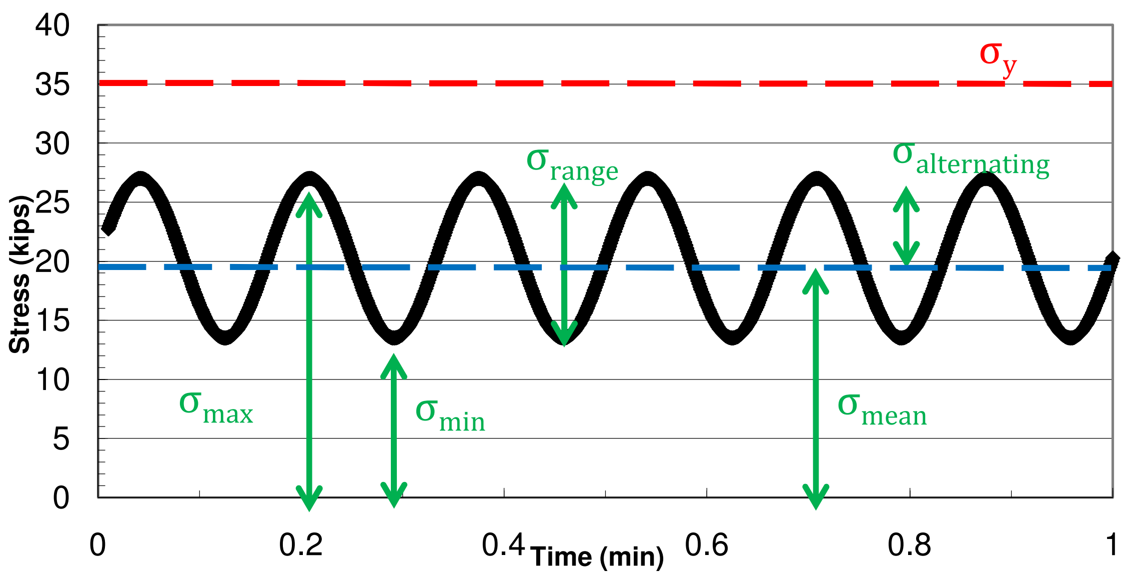

Figure 10: Ideal repeated stress cycle

Mechanical testing equipment has been selected and designated for use with the testing fixture. Testing equipment will consists of:

- MTS 55 Kip Load Frame
- MTS 55 Kip Load Cell
- MTS TestStar IIS Controller
- MTS MultiPurpose TestWare software
- Vishay 3800 Wide Range Strain Indicator (2)
- Vishay Uniaxial Strain Gauges in quarter bridge configuration (2)

The final multi-specimen fixture will incorporate five four point bend fixtures that will operate in parallel. One four-point bend fixture was machined and assembled previous to the remaining four four-point bend fixtures to evaluate the performance of the system. Two preliminary tests were run using a prelininary specimen to evaluate the capability of the system to control loading behavior and to evaluate the consistency of loading through the specimen. The experimental set-up can be seen in [Figure 11].

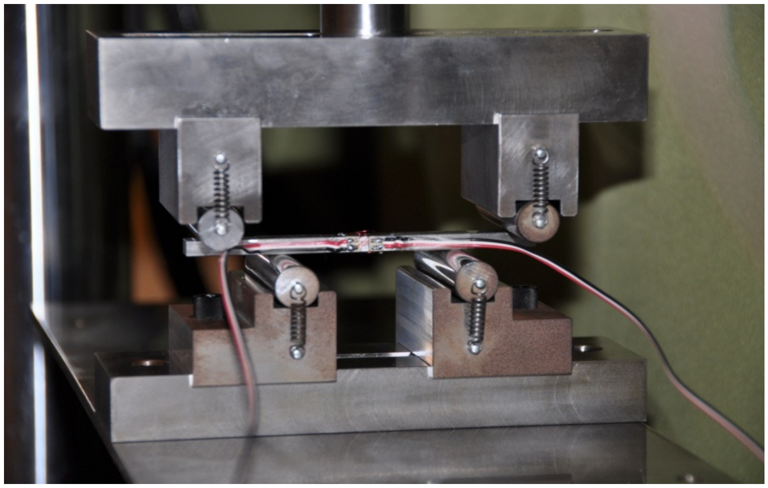

Figure 11: Single specimen four point bend fixture.

The specimen was incrementally stressed from no load to a value of that approaching the yield strength of the specimen. Data from the first test can be seen in [Figure 12]. Data from the second test can be seen in [Figure 13].

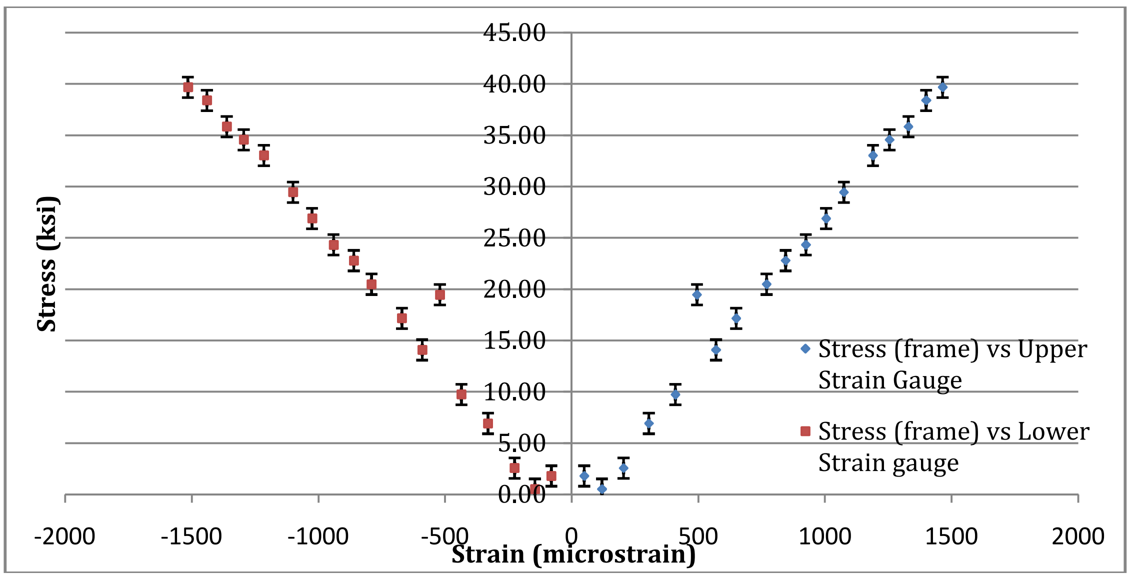

Figure 12: Stress vs. Strain in four point bending for single specimen trial 1

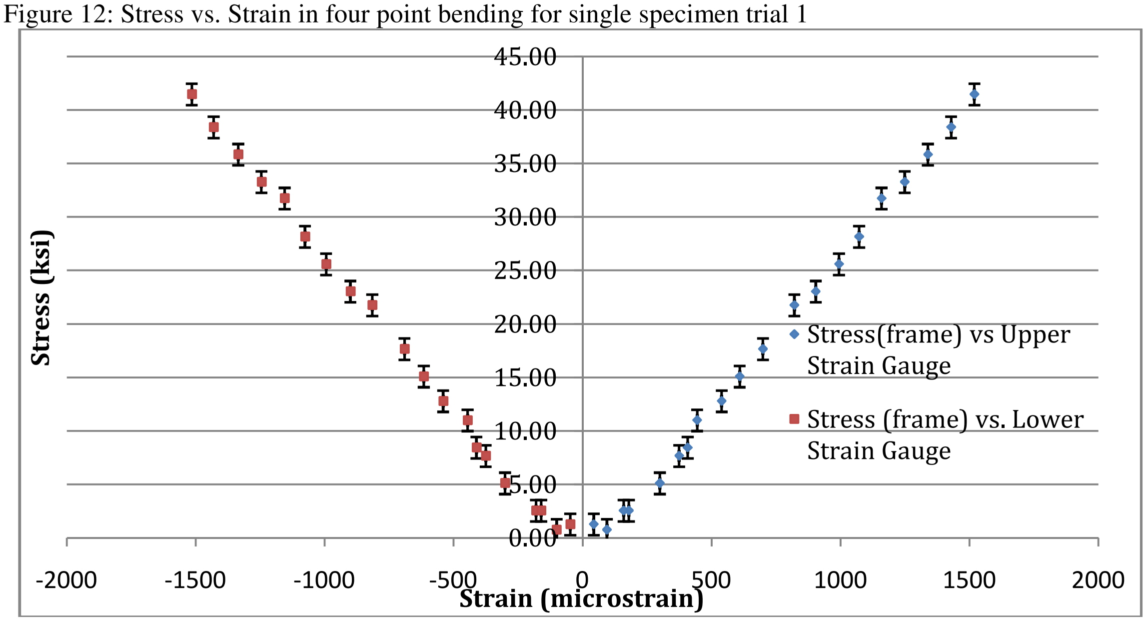

Figure 12: Stress vs. Strain in four point bending for single specimen trial 2

Evaluation of the specimen follow loading will include:

- Biological Analysis
  - Microscopy
  - 16S rRNA Gene Sequencing
- Corrosion and Cracking Characterization
  - Surface cracking
  - Pitting behavior
  - Corrosion product identification
- Electrochemical Characterization
  - Electrochemical Impedance Spectroscopy

Pitting evaluation will proceed according to standard practices from:

- ASTM G1-03: "Standard Practice for Preparing, Cleaning, and Evaluation Corrosion Test Specimens"

Table 3: Selected Cleaning Procedure from ASTM G1-03

Designation	Material	Solution	Time	Temperature
C.3.2	Iron and Steel	50g NaOH 200g granulated zinc or zinc chips,1000mL reagent water	30 to 40 min	80 to 90°C

- ASTM G46-94: "Standard Guide for Examination and Evaluation of Pitting Corrosion"
  - Density
  - Size
  - Shape
  - Corrosion product identification: EDS
- ASTM G16-95: "Standard Guide for Applying Statistics to Analysis of Corrosion data

Cracking behavior will be inspected using non-destructive penetrant methods including:

- Magnetic Particle Inspection
- Fluorescent Liquid Penetrant Inspection (LPI)
- Red Visible Liquid Penetrant Inspection (LPI)

Tensile and Compact Tension specimens

Tensile specimens were sectioned from the pipe and plate steel in the longitudinal and transverse directions to determine the extent of processing bias in the steels.

The thickness of the specimens in the transverse direction of the linepipe steels was reduced to allow for the curvature of the pipe while maintaining as much gripping surface area on the tabs as possible. There were no anomalies found in the test data to suggest that specimen gripping was cause for erroneous tensile data. Transverse specimens were sectioned and ground flat as opposed to bent to a flat condition.

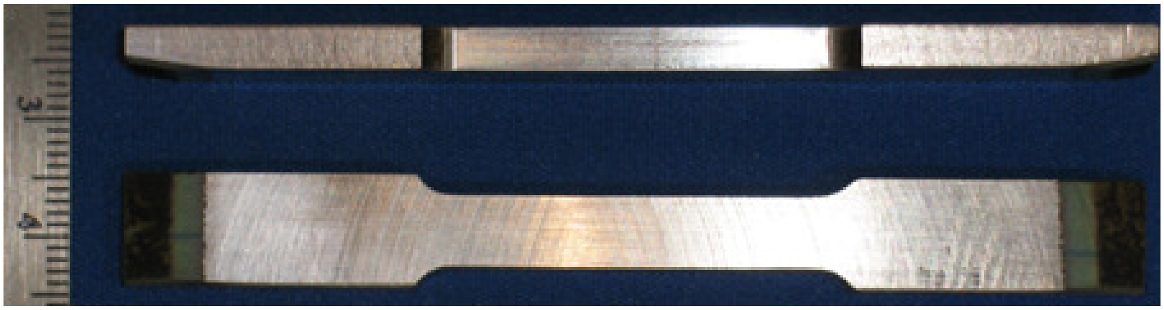

Figure 13: As received API X70 transverse ASTM E8 Sub-size specimen. The scale on the left is in millimeters. The reduction in grip area on both end tabs is clearly visible from the curvature of the pipe section.

Compact Tension [C(T)] specimens were sectioned from the linepipe and plate steel in the longitudinal directions only. The thickness in the gage section (between the loading pins) is constant but reduces beyond that to accommodate the curvature of the pipe while maximizing the plate thickness.

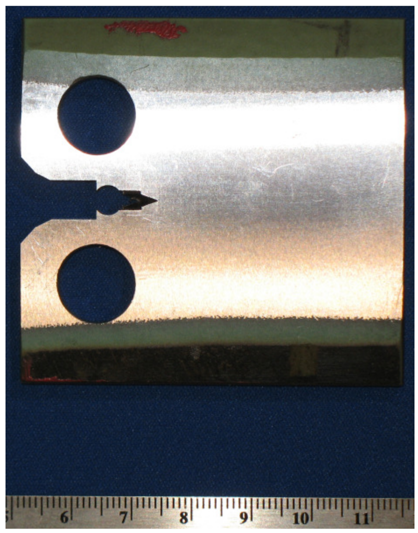

Figure 14: As received API X70 ASTM E647 specimen. The scale in the photograph is in millimeters.

Baseline Tensile Testing

Baseline tensile data was obtained from ASTM A36, API X42/52 and API X70 steel specimens. The specimens were sectioned from in-hand steel pipe and plate provided by CSM. The testing was conducted according to ASTM E8 – Standard Method for Tensile Testing of Metallic Materials. Specimens were sectioned from the pipe and plate steel in the longitudinal and transverse directions to determine the extent of processing bias in the steels. Three specimens for each processing direction and each of the steel grades were tested for a total of eighteen specimens.

This data will be used to determine the outer fiber stresses necessary for Low Cycle Bend Testing as well as K_{Imax} values necessary for Fatigue Crack Growth Rate Testing. The tensile tests were performed at room temperature, in air at an initial engineering strain rate of 1.6×10^{-3} s^{-1} up to 0.02 strain at which point the actuator velocity was increased by an order of magnitude to shorten the test time (in accordance with E-8). Yield strength measurements were performed using the 0.2 pct. offset strain measurement described in E-8, these data are used to determine loading conditions for subsequent tests, but it should be noted that API specifies yield strength based on a Elongation Under Load (EUL) condition (typically at 0.5 pct. strain) for pipeline grades.

First annual review meeting

The first annual review meeting was held at the Colorado School of Mines on September 15, 2009. Attendees included joint industry partners, representatives of the DOT-PHMSA and researchers from CSM, NIST and iCorr. Meeting agenda, presentations, and minutes will be uploaded as supporting documents with this report.

Results and Conclusions

Additional contacts were established with personal involved with the production, transportation and storage of ethanol fuels. Field trips were made to conduct sampling and gather information about handling of ethanol fuels. Common field practices as well as challenges were noted and discussed. Arrangements were made for ongoing field sampling with existing contacts.

Biological analyses was conducted to determine the microbial species associated with fuel ethanol infrastructure and to investigate how this microbial diversity may affect corrosion behavior of the steel. Samples taken from filters at a fuel ethanol production facility have been analyzed via 16S rRNA gene sequencing. 16S rRNA gene sequencing has identified spore-forming bacteria.

Cultivation experiments to identify microbes capable of surviving ethanol fuel environments. Viable organisms have been cultivated from the ethanol fuel filters. The identification of viable microbes isolated from ethanol fuel blend infrastructure indicates that corrosion-causing microbes may survive harsh conditions and thrive when the environment changes resulting in enhanced corrosion, which may occur during batch flow of various fuels.

The spores of some Bacillus sp. have the ability to promote the oxidation of manganese. These spores may promote MIC. Investigations to determine if spores can oxidize manganese and promote corrosion in ethanol fuel blend environments has begun.

Electrochemical data was collected and analyzed to characterize corrosion behavior of ASTM-A36 steel in ethanolic solutions of varying oxygen and water concentrations.

Testing parameters for low cycle bend testing have been established. A multi-chambered four-point bend fixture has been constructed. A trial four-point bend fixture was assembled on a loading frame at NIST, Boulder. Two trials were run to evaluate the performance of a single four-point bend fixture

The baseline tensile testing is complete, however, final data reduction and analysis is in progress at the time of this writing. No data anomalies were found. It is expected that a full report on the results and conclusions of that testing will be available for publication in the next quarterly report.

Issues, Problems or Challenges

Further characterization of microbiological diversity associated with ethanol fuel infrastructure is necessary.

Further work is necessary to determine growth conditions and cultivate microbial species procured from field sampling in laboratory environments.

Refinement of electrochemical techniques to better acquire electrochemical measurements in low solution conductivity is required.

The baseline tensile testing was performed without any issues to suspect the validity and application of the data to the steel grades pertinent to this program.

Test frames are being setup to mitigate the hazard potential of testing in fuel mixture environments. No problems are identified at this point as potential causes for delay in the program. The challenges associated with this testing are anticipated to include multi-chamber alignment as well as handling and disposal of the hazardous fuel mixtures required for the program. These challenges have administrative as well as engineering controls to ensure safe and reliable execution of the test program.

Fatigue Crack Growth Rate testing is the next test program for NIST to accomplish for each of the steel grades. To date the test matrix is to be determined with respect to the electrochemical and biological environments. For each steel grade the matrix allows for a maximum of six test specimens. The equipment identified for testing requires additional hardware which is on order with an anticipated delivery date sixty days from this writing. The challenges associated with this testing are anticipated to also include handling and disposal of the hazardous fuel mixtures required for the program. These challenges have administrative as well as engineering controls to ensure safe and reliable execution of the test program.

Plans for Future Activity

Conduct further thin-film and growth experiments to determine the potential for microbes to survive in high ethanol environments and the potential for spore-forming microbes to effect corrosion.

Continue to analyze 16S rRNA gene data as well as conduct cultivation experiments to support field assessment.

Cultivated microbes on bend specimen prior to cyclic loading.

Coat, assemble, and calibrate the multi-specimen cyclic-loading bending fixture and bath assembly.

Further develop and refine a MIC/ethanol review paper.

Characterize the role of variables such as salts, gasoline, denaturants and microbes on the corrosion behavior of steel using electrochemical impedance spectroscopy (EIS), to select environments for mechanical testing.

Perform in-situ electrochemical analysis on the cyclically loaded steel specimens in ethanol-gasoline mixtures both without and with microbes.